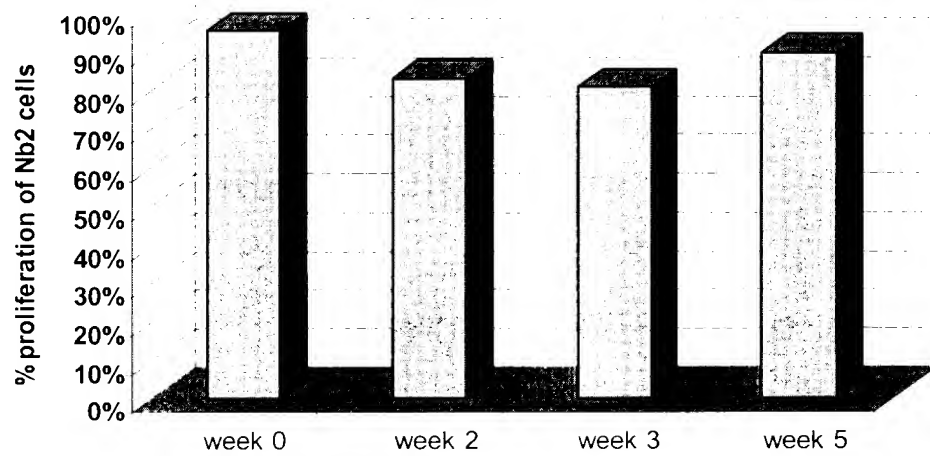


1/18

**Stability of HA-hGH at 37°C in cell culture media**

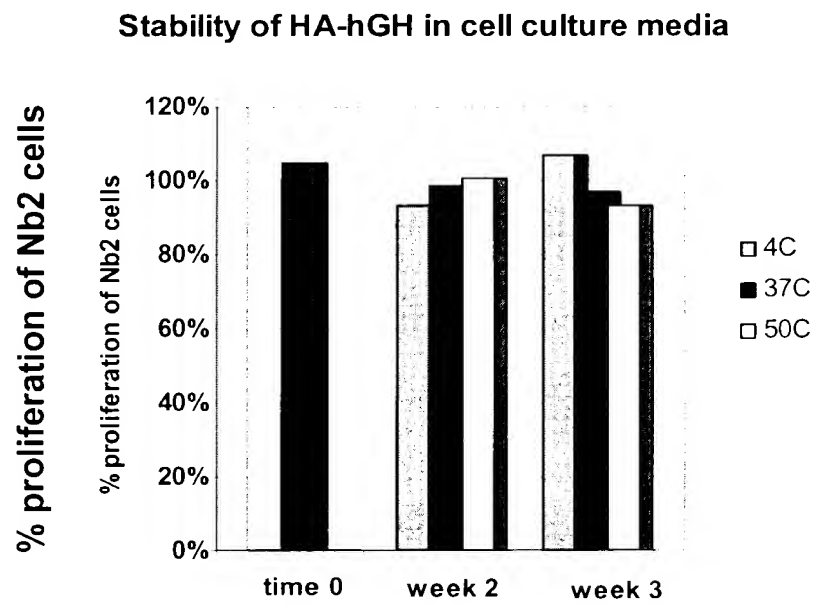


2ng/ml HA-hGH

hGH has no activity by week 2

**Figure 1**

2/18



60ng/ml of HA-hGH

**Figure 2**

3/18

### Nb2 Cell Proliferation Assay (24hrs)

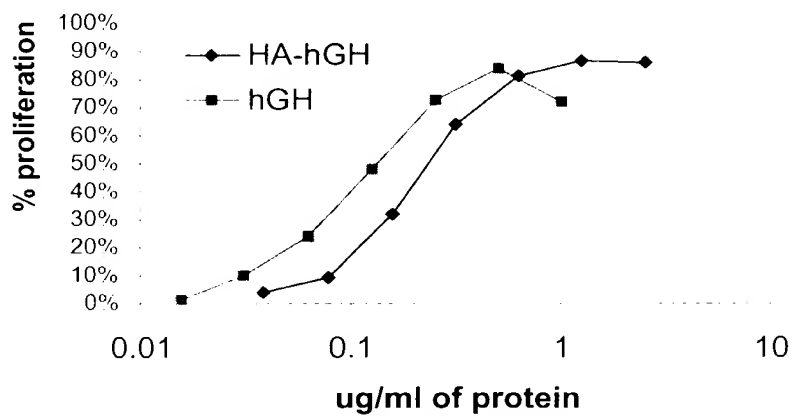


Figure 3A

### Nb2 Cell Proliferation Assay (48hrs)

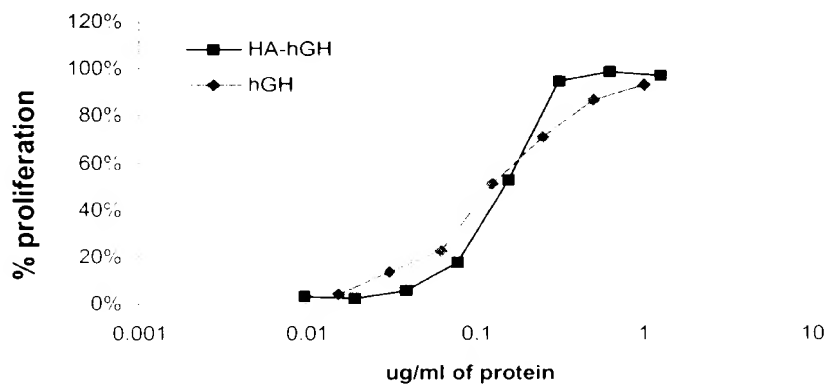


Figure 3B

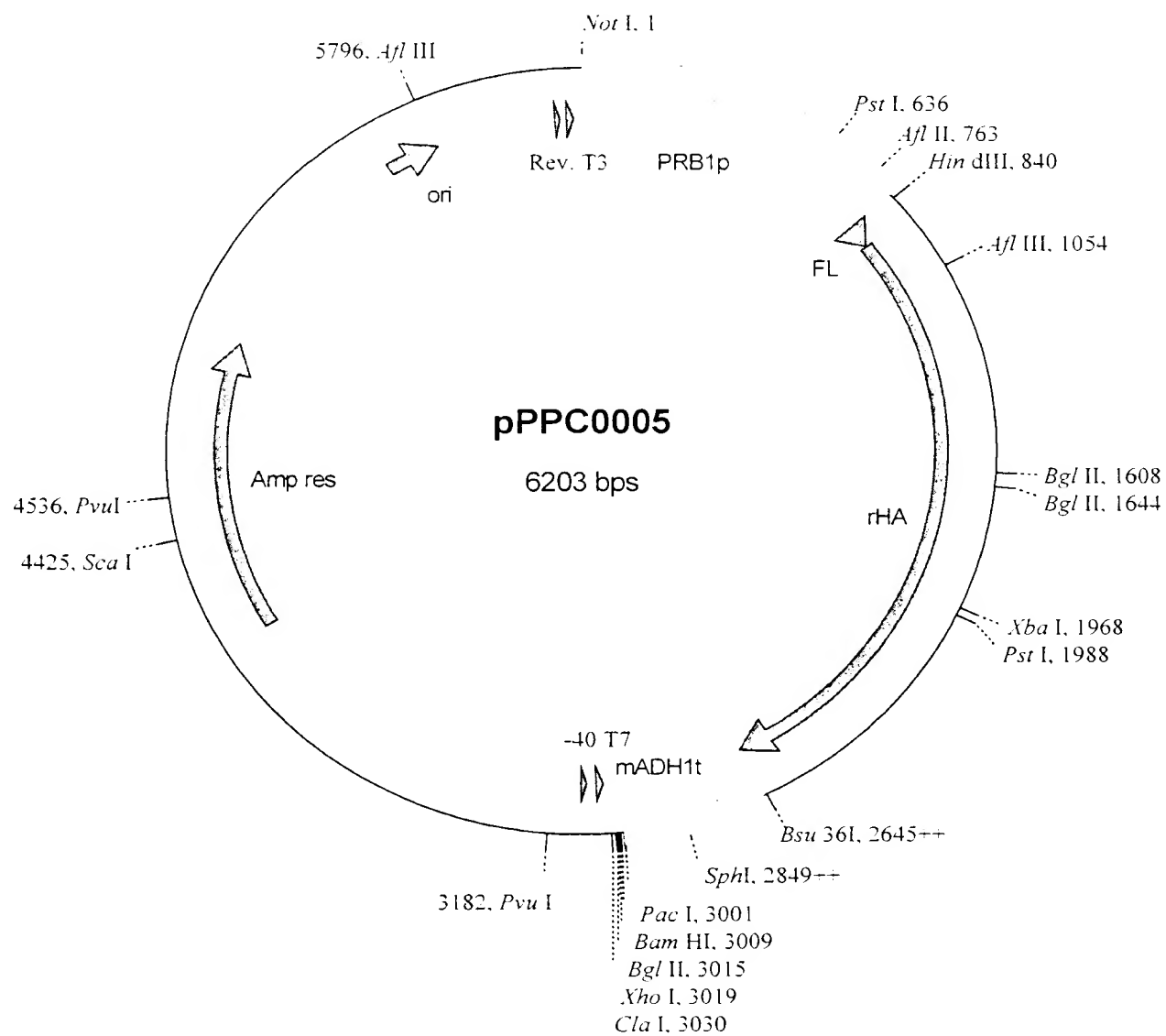


Figure 4

5/18

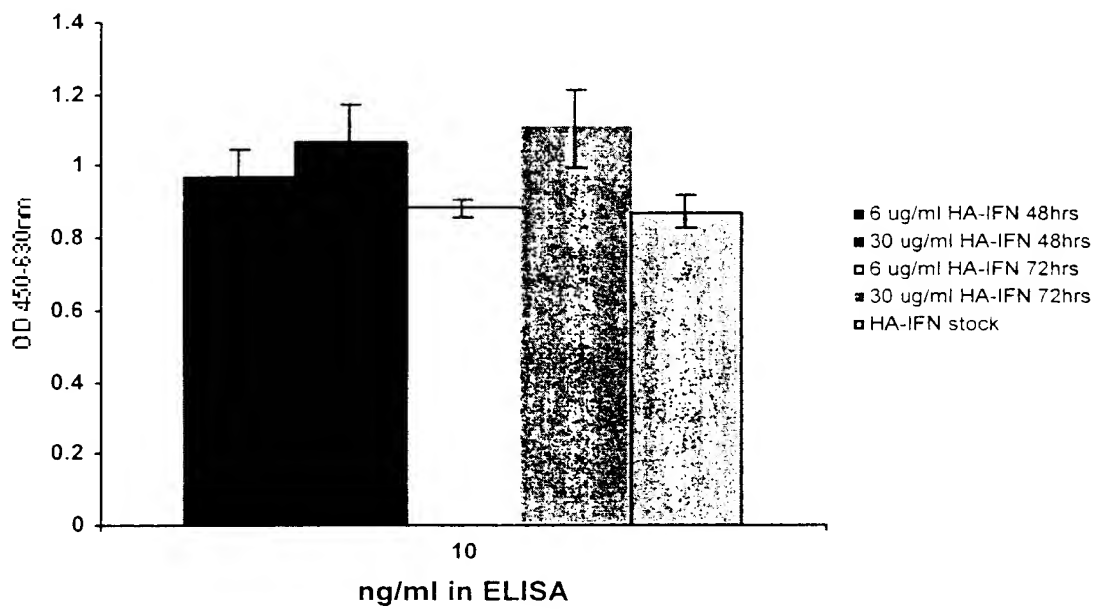


Figure 5

Figure 6

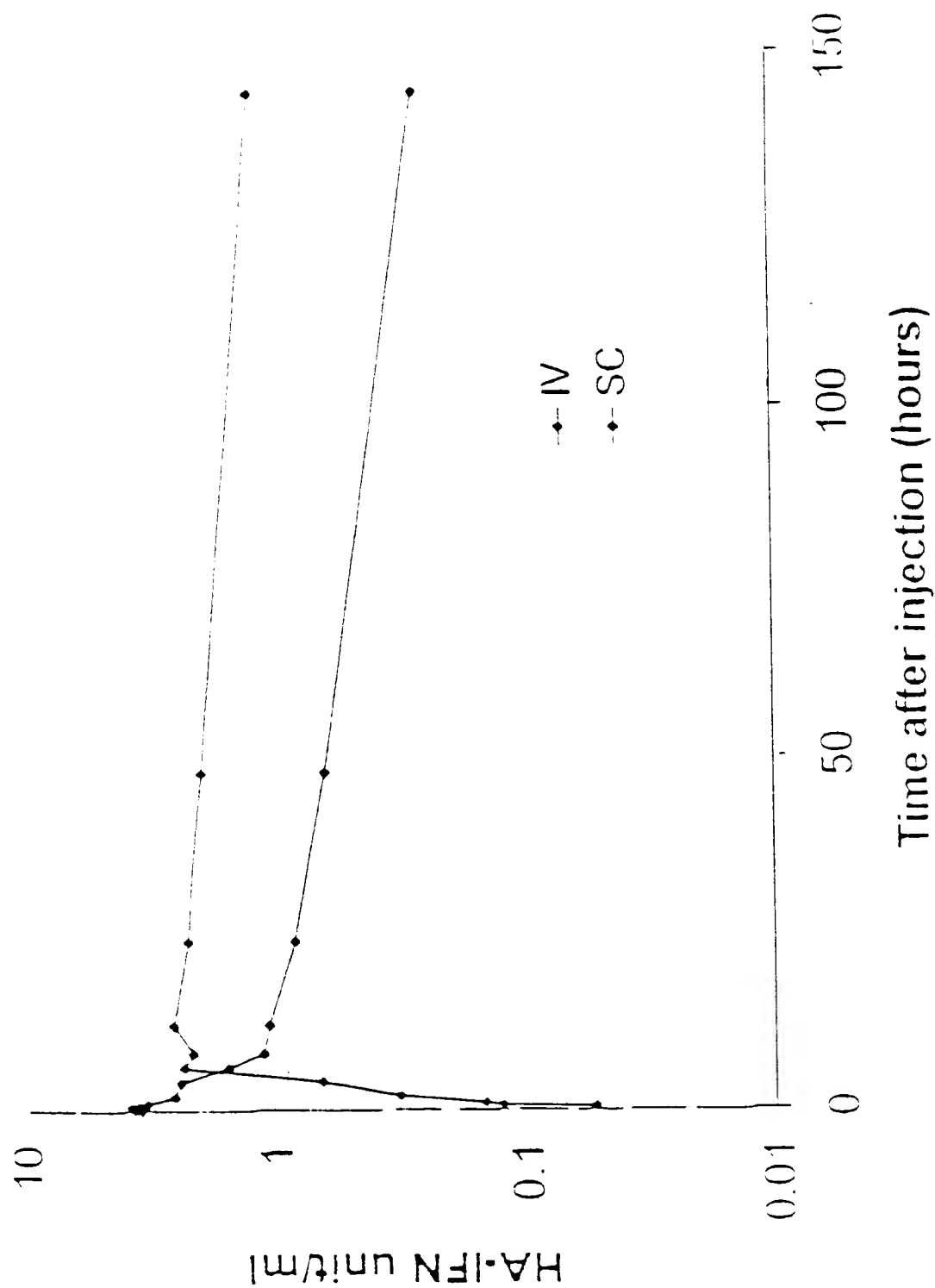
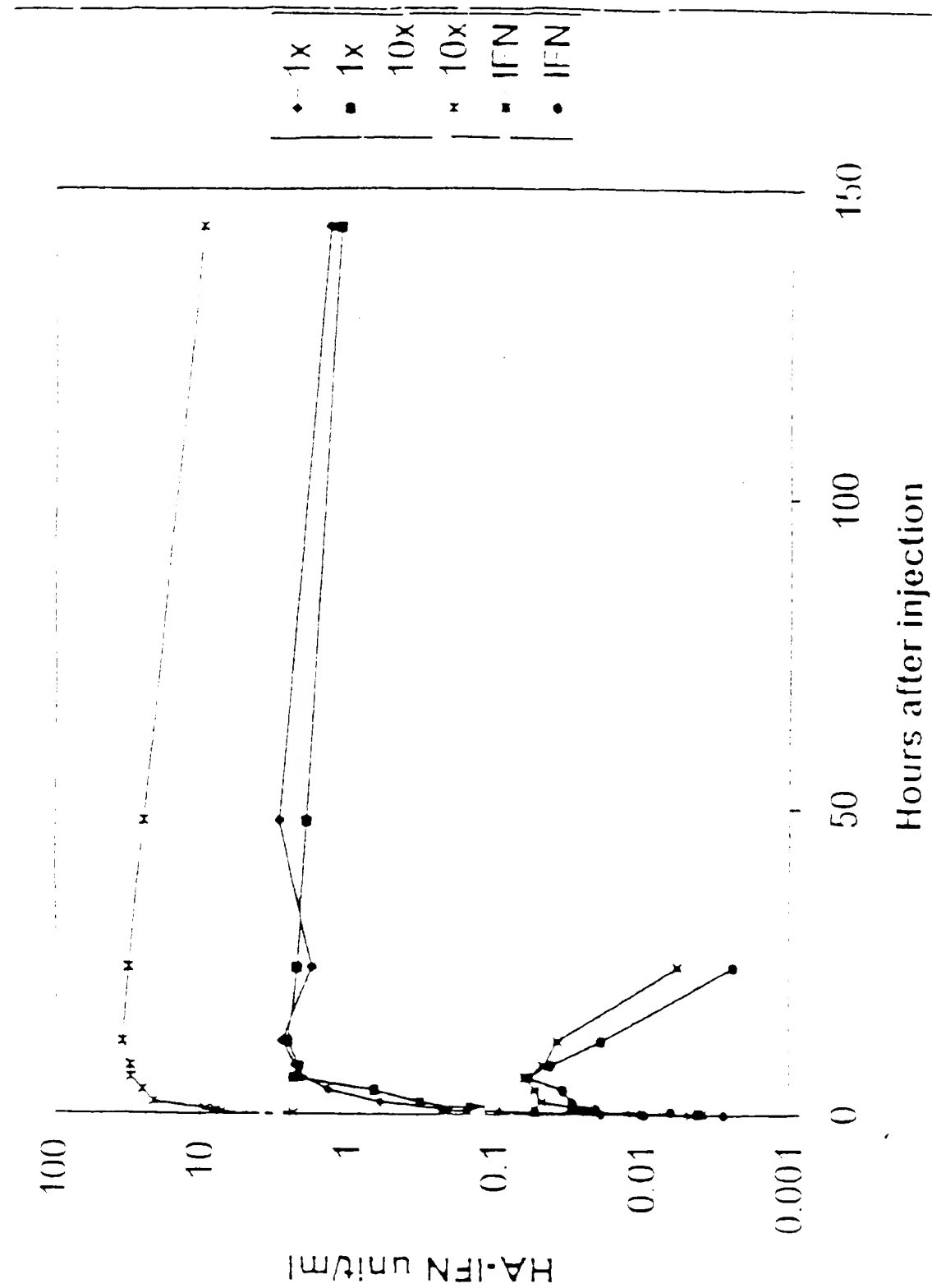
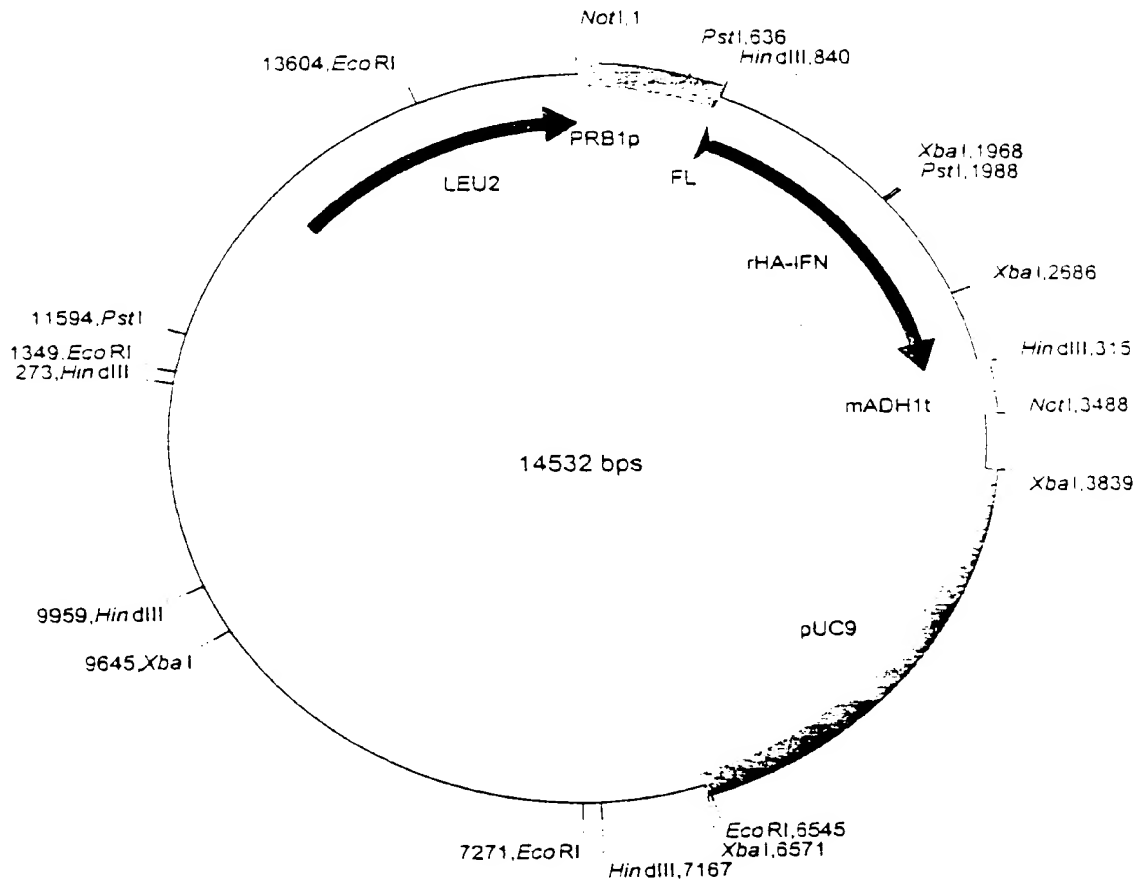


Figure 7





**Figure 8.** The HA-IFN $\alpha$  expression cassette in pSAC35. The expression cassette comprises  
 PRB1 promoter, from *S. cerevisiae*.  
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF $\alpha$ -1 leader.  
 HA-IFN $\alpha$  coding sequence with a double stop codon (TAATAA)  
 ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the Hind III/BamHI fragment generally used.

**Figure 8**



9/18

**Localisation of 'Loops' based on the HA Crystal Structure**  
**which could be used for Mutation/Insertion**

```

1    DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQCCPFEDHV KLVNEVTEFA
      HHHHH HHH      HHH HHHHHHHHHHH      HHHHH HHHHHHHHHHH

      I                      II                      III
51   KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
      HHHHH      HHHHH HHHHH      HHHH H      HHHH

101  CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
      HHHH      H      HHHHHHHHH      HHHHHHHHH HHHHH

      IV
151  APELLFFAKR YKAAFTECCO AADKAACLLP KLDELFDDEGK ASSAKQRLKC
      HHHHHHHHHHH HHHHHHHHH      HHHHH HHHHHHHHHHH HHHHHHHHHHH

      V
201  ASLQKFGEPA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
      HHHHH      HH HHHHHHHHHHH HH      HHH HHHHHHHHHHH HHHHHH HH

      VI                      VII
251  LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
      HHHHHHHHHHH HHHHH      HHHHH      HHHHHHH H

301  DLPSLAADFV ESFDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLLA
      HHHH      HHHHHH      HHHHHHH HHHHHH      HHHHHHHHH

      VIII
351  KTYETTTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
      HHHHHHHHHHH      HH      H      HHHHH HHHHHHHHHHH HHHHHHH

      IX
401  YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
      HHHHHHHHHHH HHHH      H HHHHHHHHHHH      HHH      HHHHHHHHH

      X                      XI
451  DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
      HHHHHHHHHHH HHHHH      HHHHHHHHHH      HHHHHHHH

501  EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
      HHH      HHH HHHHMMEHHH HHH      HHHHHHHHH

      XII
551  FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL
      HHHHHHHHH      HHHH HHHHHHHHHHH HH

```

Loop	Loop
I Val54-Asn61	VII Glu280-His238
II Thr76-Asp89	VIII Ala362-Glu368
III Ala92-Glu100	IX Lys439-Pro447
IV Gln170-Ala176	X Val462-Lys475
V His247-Glu252	XI Thr478-Pro486
VI Glu266-Glu277	XII Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV  
 151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGF ASSAKQRLKC  
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHH

IV  
 151 APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDEL RDEGF ASSAKQRLKC  
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

**b. Insertion (or replacement) of Randomised sequence into Loop IV.**

(X)<sub>n</sub>



IV

151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGF ASSAKQRLKC  
 HHHHHHHHHH HHHHHHHHHH HHHHH HHHHHHHHHHH HHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

**Figure 10**

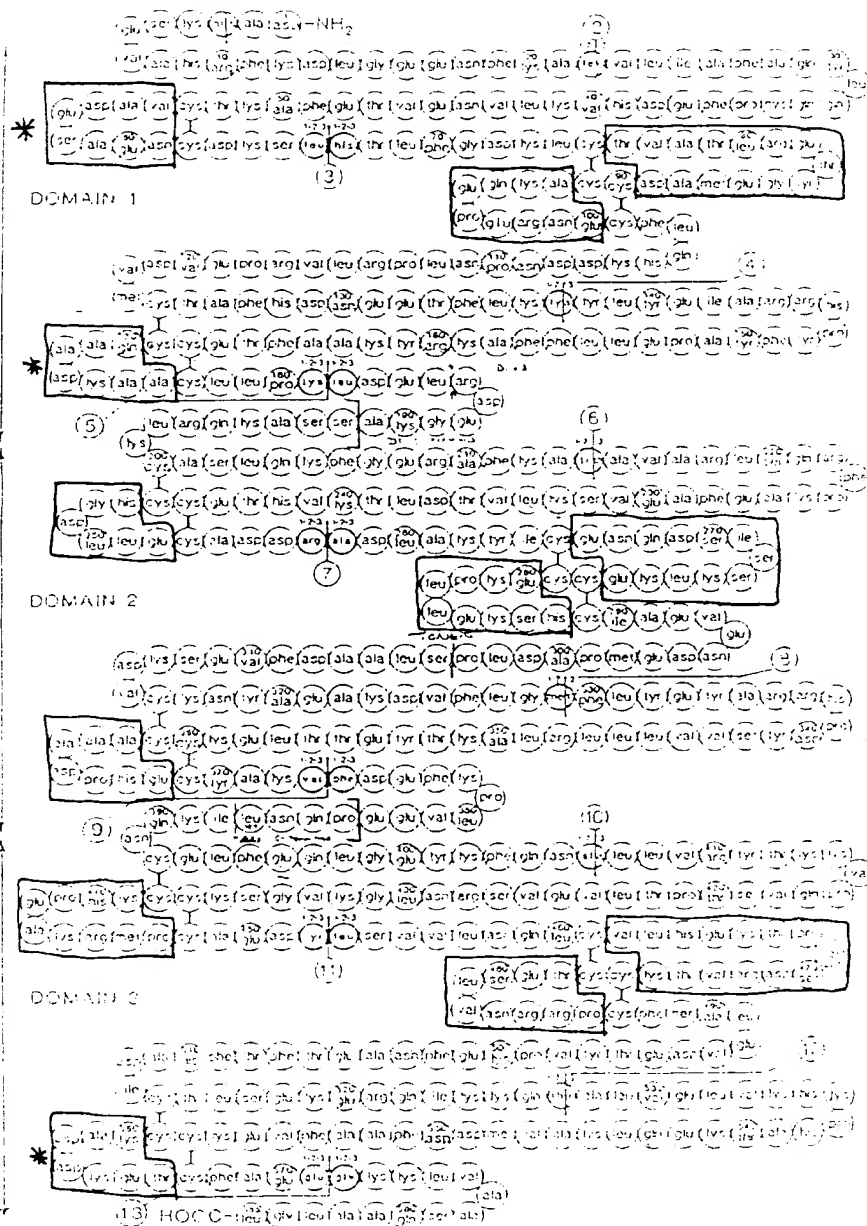


Figure 11



Disulfide bonds shown in yellow

**Figure 12: Loop IV Gln170-Ala176**

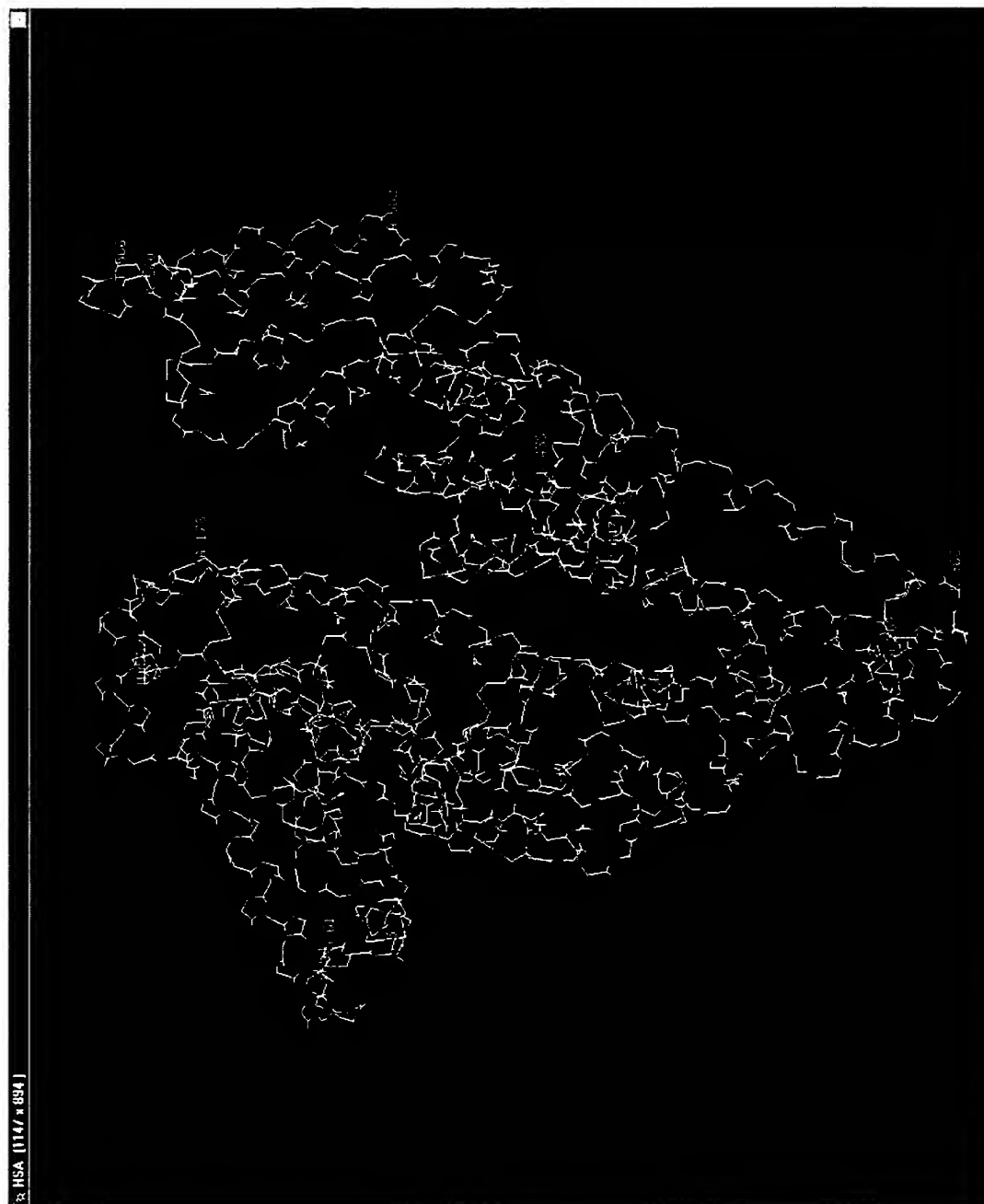
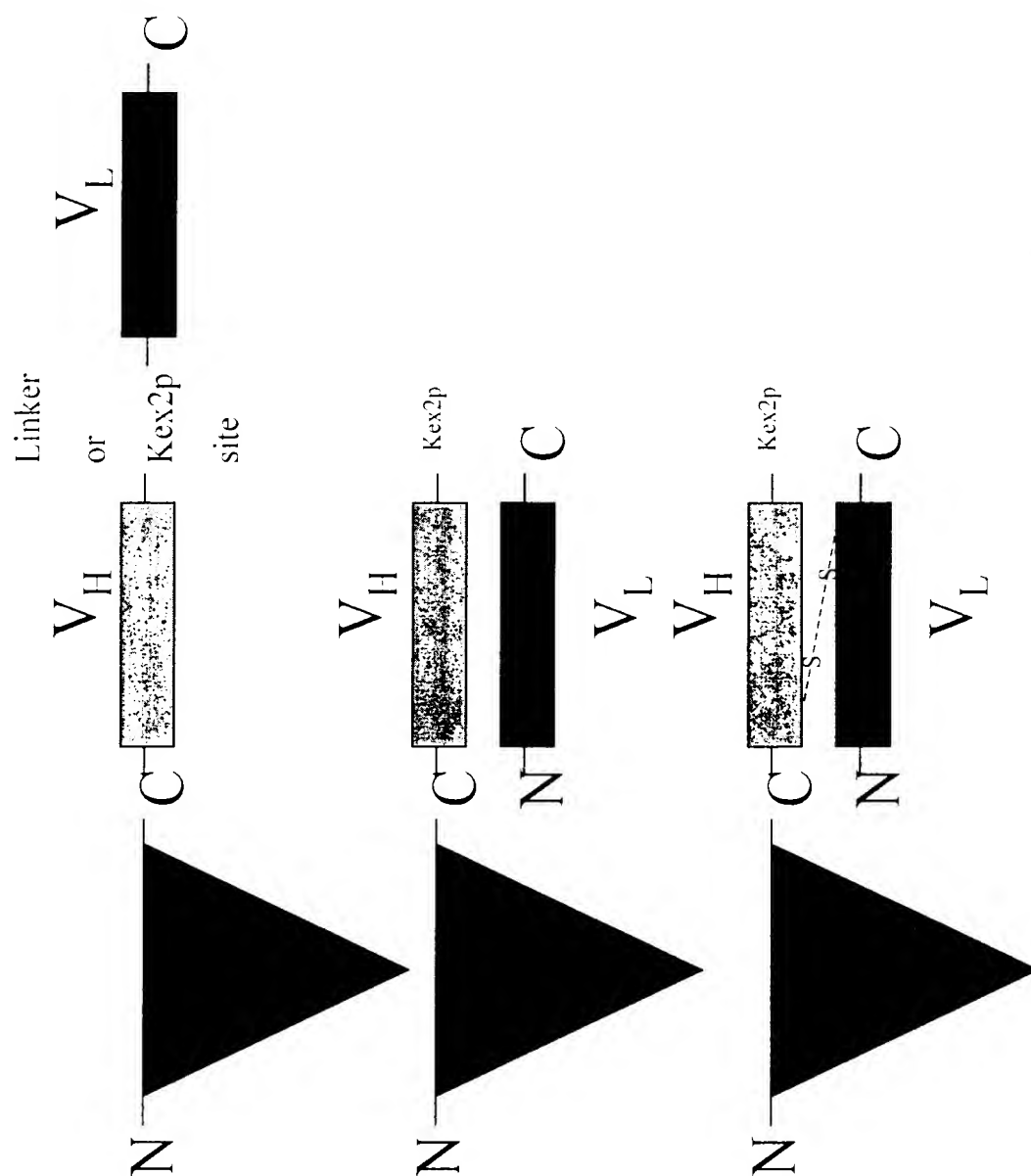


Figure 13: Tertiary Structure of HA



**Figure 14: Schematic Diagram of Possible ScFv Fusions**  
(Example is of a C-terminal fusion to HA)

15/18

1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60  
 1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120  
 21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT CAT GAG TCA GAT GAA 180  
 41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240  
 61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300  
 81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TAC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360  
 101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420  
 121 D V M C T A F H D N E E T F L K K Y L Y 140

421 GAA ATT GCT AGA AGA CAT CTT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480  
 141 E I A R R H P Y F Y A P E L L L F F A K R 160

Figure 15A

# 16/18

481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GGC TGC CTG TTG CCA 540  
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GGC AAA CAG AGA CTC AAA TGT 600  
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GGC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660  
201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CTT AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720  
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780  
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840  
261 A K Y I C E N Q D S I S S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GGC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900  
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GGT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960  
301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B



1021 TAC TCT GTC GTG CTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080  
341 Y S V V L L L R L A K T Y E T T L E K C 360

1981 TTT GGC GCT GGA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTT GAT GAA TTT AAA CCT CTT 1140  
361 C A A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CTT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200  
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1291 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CUC CAA GTC TCA ACT 1260  
401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTG TCA AGA AAC CTA GGA AAA GTG GGC AAG TGT TGT AAA CAT 1320  
421 P T L V E V S R N L G K V G S K C G K H 440

1321 CTT GAA GCA AAA AAG ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380  
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TCC ACA GAG TCC 1440  
461 C V L H E K T P V S D P V T K C C C E S 480

**Figure 15C**

# 18/18

1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GGT CTG GAA GTC GAT GAA ACA TAC GGT CCC AAA 1500  
 481 L V N R R P C F S A L E V D E T Y V P K 500  
  
 1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560  
 501 E F N A E T F T F H A D I C T L S E K E 520  
  
 1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620  
 521 R Q I K K Q T A L V E L V K H K P K A T 540  
  
 1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680  
 541 K E Q L K A A V M D D F A A F V E K C K 560  
  
 1681 GGT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740  
 561 A D D K E T C F A E E G K K L V A A S Q 580  
  
 1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782  
 581 A A L G L \* 585

Figure 15D